



# Biology of Blood and Marrow Transplantation

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## Comparison of Chimerism and Minimal Residual Disease Monitoring for Relapse Prediction after Allogeneic Stem Cell Transplantation for Adult Acute Lymphoblastic Leukemia



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### ABSTRACT

Little data are available on the relative merits of chimerism and minimal residual disease (MRD) monitoring for relapse prediction after allogeneic hematopoietic stem cell transplantation (HCT). We performed a retrospective analysis of serial chimerism assessments in 101 adult HCT recipients with acute lymphoblastic leukemia (ALL) and of serial MRD assessments in a subgroup of 22 patients. All patients had received myeloablative conditioning. The cumulative incidence of relapse was significantly higher in the patients with increasing mixed chimerism (in-MC) compared with those with complete chimerism, low-level MC, and decreasing MC, but the sensitivity of in-MC detection with regard to relapse prediction was only modest. In contrast, MRD assessment was highly sensitive and specific. Patients with MRD positivity after HCT had the highest incidence of relapse among all prognostic groups analyzed. The median time from MRD positivity to relapse was longer than the median time from detection of in-MC, but in some cases in-MC preceded MRD positivity. We conclude that MRD assessment is a powerful prognostic tool that should be included in the routine post-transplantation monitoring of patients with ALL, but chimerism analysis may provide additional information in some cases. Integration of these tools and clinical judgment should allow optimal decision making with regard to post-transplantation therapeutic interventions.

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### INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HCT) is a well-established postremission therapy in adults with acute lymphoblastic leukemia (ALL) with certain high-risk features [1,2]. Some protocols advocate HCT even in standard-risk ALL [3,4]. HCT is also applied in patients in second or higher complete remission (CR), and for selected patients with refractory disease. Although treatment-related mortality has declined in recent years, relapse remains a frequent cause of death after HCT, particularly in patients with advanced disease [5].

Salvage therapies for overt hematologic relapse after HCT are often futile [6], but intervention in early relapse

when the leukemia burden is still low may be effective [7,8]. The 2 methods available for early relapse prediction are monitoring of minimal residual disease (MRD), by flow cytometry for leukemia-associated aberrant immunophenotypes or by real-time quantitative polymerase chain reaction (qPCR) for fusion genes or clonal immunoglobulin or T cell receptor (Ig/TCR) gene rearrangements [9–17], or sequential measurements of donor chimerism, primarily by fluorescence-based PCR amplification of short tandem repeat (STR) markers [18–22]. Whereas MRD directly detects residual or reemerging leukemia clones, chimerism analysis provides information only on the persistence or reemergence of autologous hematopoiesis. In addition, standard MRD assays usually have at least a 1 log greater sensitivity than assays used for the assessment of chimerism [18,23].

Both of these methods have been applied in the post-transplantation setting in adults with ALL [19], but their relative merits with regard to relapse prediction have not been thoroughly evaluated. The impact of chimerism has been analyzed primarily in pediatric ALL populations

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[8,19–21,24–27], in whom distinct immunologic mechanisms may exist [28]. MRD studies, although indicating an increased relapse risk in patients with MRD positivity after HCT [7,11,29–33], generally have not provided comparative data to chimerism. In this context, a recent National Cancer Institute workshop concluded that it is critically important to assess the clinical relevance of MRD and chimerism surveillance in individual diseases [19], and a recent evidence-based review on the role of HCT in adult ALL concluded that monitoring of MRD after allogeneic HCT is an area meriting further research [34].

Here we present a retrospective analysis of serial chimerism assessments in 101 adults with ALL, and of serial MRD assessments in a subgroup of 22 patients, all of whom had undergone myeloablative allogeneic HCT at our center between 1999 and 2009. We examined the kinetics of MRD and chimerism signals and evaluated whether either of these 2 methods provides better relapse prediction, potentially allowing for earlier and more successful therapeutic intervention.

## PATIENTS AND METHODS

### Patients

A total of 146 adults with ALL underwent allogeneic HCT at our center between 1999 and 2009. For the present study, patients who received reduced-intensity conditioning and/or underwent a second allogeneic HCT ( $n = 31$ ), as well as patients for whom chimerism data were unavailable (owing to, eg, death before day 28 or follow-up at external centers [ $n = 14$ ]), were excluded, leaving a final study population of 101 patients (Table 1). MRD data were available for a subgroup of 22 patients.

All patients were diagnosed and treated under the German Multicenter Study Group for Adult ALL (GMALL) study protocol 06/99 or 07/03 [1,35,36]. Patient-, disease-, and transplantation-related characteristics and follow-up information were recorded prospectively in an electronic database, and chimerism and MRD data, as well as data on the occurrence of graft-versus-host disease (GVHD), were gathered retrospectively from patient files by standardized medical record abstraction. All patients gave informed consent for analysis of their data, in accordance with the Declaration of Helsinki.

The patients were at high risk for relapse or progression and underwent transplantation in first CR ( $n = 59$ ), in second CR or greater ( $n = 19$ ), in active relapse ( $n = 21$ ), or with primary refractory disease ( $n = 2$ ) (Table 1).

### Conditioning and GVHD Prophylaxis

Patients received myeloablative conditioning based on total body irradiation (TBI)  $6 \times 2$  Gy, in combination with either cyclophosphamide  $2 \times 60$  mg/kg ( $n = 60$ ) or etoposide  $1 \times 60$  mg/kg ( $n = 33$ ), cyclophosphamide  $2 \times 50$  mg/kg + etoposide  $1 \times 50$  mg/kg ( $n = 5$ ), or an alternative regimen without TBI based on treosulfan  $3 \times 12$  g/m<sup>2</sup> + cyclophosphamide  $2 \times 60$  mg/kg + etoposide  $1 \times 30$  mg/kg ( $n = 2$ ) or busulfan  $4 \times 4$  mg/kg + cyclophosphamide  $2 \times 60$  mg/kg (Table 1). GVHD prophylaxis consisted of cyclosporine A (CSA) combined with short-course methotrexate ( $n = 76$ ), mycophenolate mofetil ( $n = 9$ ), or prednisolone ( $n = 16$ ). Antithymocyte globulin (ATG; Fresenius)  $3 \times 10$  or  $20$  mg/kg/day was given initially only to patients with mismatched donors and then, starting in 2004, also in patients with a matched unrelated donor ( $n = 29$ ).

### Allogeneic Transplantation

HLA typing was performed by PCR sequence-specific primer low-resolution typing for HLA class I and genomic high-resolution DNA-based typing for HLA class II. Stem cell source was either bone marrow ( $n = 9$ ) or peripheral blood stem cells ( $n = 92$ ) from HLA-matched related donors ( $n = 37$ ), HLA-matched unrelated donors ( $n = 52$ ), or HLA-mismatched donors ( $n = 12$ ) (Table 1). Leukocyte engraftment was defined as the first of 3 consecutive days with a leukocyte count  $>1000$   $\mu$ L and/or a neutrophil count  $>500$   $\mu$ L. Platelet engraftment was defined as the first of 3 consecutive days with a platelet count  $>20,000$   $\mu$ L without transfusions. Acute GVHD (aGVHD), late aGVHD, and chronic GVHD (cGVHD) were classified according to the National Institutes of Health consensus criteria [37], as described previously [38].

### Donor Lymphocyte Infusion

Donor lymphocyte infusion (DLI) were administered to 38 patients according to a standard protocol [38,39] in a preemptive manner for patients with persistent or recurrent mixed chimerism ( $n = 16$ ) or MRD ( $n = 2$ ), in a

**Table 1**

Patient and Treatment Characteristics

Characteristic	Value
Number of patients	101
Sex, %	
Male	64
Female	36
Age at HCT, yr, median (range)	33 (17–55)
Karnofsky Performance Status, %	
100	49
90	41
$\leq 80$	10
HCT-CI, %	
Low	28
Intermediate	46
High	27
Disease status at HCT, %	
CR1	58
CR2 or greater	19
No CR	23
Lineage, %	
B-ALL	71
T-ALL	29
Cytogenetic risk group, %	
Philadelphia chromosome positive	22
High risk [t(4;11), t(8;14), CA]	16
Other aberrations	16
Normal karyotype	47
Donor type, %	
Matched related donor	37
Matched unrelated donor	51
Mismatched donor	12
Stem cell source, %	
Peripheral blood stem cells	91
Bone marrow	9
ATG during conditioning, %	
Yes	29
No	71
DLI given, %	
Yes	38
No	62

CA indicates complex aberrant.

Percentages may not total 100% because of rounding.

prophylactic manner for patients with active disease at transplantation ( $n = 13$ ), or in a therapeutic manner for morphological relapse after salvage chemotherapy ( $n = 7$ ). DLI was given at 4-week intervals in escalating doses (related donor:  $1 \times 10^7$ ,  $5 \times 10^7$ , and  $1 \times 10^8$  CD3<sup>+</sup> cells/kg body weight; unrelated donor:  $5 \times 10^6$ ,  $1 \times 10^7$ ,  $5 \times 10^7$ , and  $1 \times 10^8$  CD3<sup>+</sup> cells/kg body weight) after previous discontinuation of immunosuppression and only in the absence of GVHD and infection.

### Chimerism and MRD Analysis

Chimerism analysis was performed at the Institute of Legal Medicine and Forensic Sciences, Charité—University Medicine Berlin, and determined by a multiplex STR assay with 9 STR systems plus the amelogenin gene with fluorescence-based detection. The analysis included CD4<sup>+</sup>, CD8<sup>+</sup>, and CD19<sup>+</sup> cells in peripheral blood and CD34<sup>+</sup> cells in bone marrow, at a sensitivity of  $10^{-2}$  [40]. Chimerism analysis was performed routinely at 1, 3, 6, 9, 12, 18, 24, 36, 48, and 60 months after transplantation. Patients with no signal of host DNA during follow-up were categorized as having complete donor chimerism (CC), whereas patients with a measurable host signal but not more than 5% at any time point were categorized as low-level mixed chimerism (ll-MC). Patients with a  $\geq 5\%$  increase in autologous cells between 2 consecutive assessments were classified as having increasing mixed chimerism (in-MC), whereas patients with a host signal immediately post-transplantation that decreased spontaneously were categorized as having decreasing mixed chimerism (de-MC) [20].

MRD analysis was performed at the GMALL study group central MRD laboratories, as described in detail elsewhere [16,35,41,42]. In brief, for BCR-ABL–positive ALL, complementary DNA was synthesized from RNA and BCR-ABL qPCR was performed, using ABL as the housekeeping gene [42]. For BCR-ABL–negative ALL, clonal Ig/TCR gene rearrangements were identified by gene scanning and/or heteroduplex gel analysis and then sequenced. MRD was then determined by clone-specific qPCR, with the aim of using 2 molecular markers with a sensitivity of at least  $10^{-4}$ . MRD relapse was

defined as reappearance of MRD within the quantitative range ( $\geq 10^{-4}$ ) after previous achievement of MRD negativity [16,35,41]. MRD assessments were done in bone marrow and scheduled for the same intervals as chimerism analysis.

### Statistics

Overall survival (OS) was estimated by the Kaplan-Meier method and compared using the log-rank test. The cumulative incidences of relapse and nonrelapse mortality (NRM) were estimated taking into account competing risks and compared using Gray's test. The prognostic impact of chimerism and MRD on relapse, NRM, and OS was estimated using multivariate Cox proportional hazards regression analysis, with chimerism and MRD status introduced as a time-dependent covariate [43], in addition to the baseline characteristics of year of transplantation, sex, age, Karnofsky Performance Status score, HCT-specific comorbidity index (HCT-CI), disease status, lineage, cytogenetic risk group, donor type, stem cell source, use of ATG, and the time-dependent covariates administration of DLI and occurrence of aGVHD, late aGVHD, and cGVHD. Sensitivity and specificity of chimerism and MRD assessment with regard to relapse detection were assessed as well. Results of comparative tests were considered significant at a 2-sided  $P$  value  $< .05$ . Statistical analyses were performed using SPSS 20.0 (IBM, Armonk, NY), NCSS 9 (NCSS, Kaysville, UT), and the *cmprsk* package in R (R Foundation for Statistical Computing, Vienna, Austria).

## RESULTS

### Overall Outcome

Leukocyte engraftment occurred a median of 16 days post-transplantation (range, 10 to 32 days), and platelet engraftment occurred at a median of 19 days (range, 9 to 82 days). After a median follow-up in surviving patients of 74 months (range, 13 to 140 months), projected OS was 83% (95% confidence interval [CI], 76% to 90%) at 1 year, 67% (95% CI, 58% to 76%) at 2 years, 60% (95% CI, 50% to 70%) at 3 years, and 56% (95% CI, 47% to 66%) at 5 years. The cumulative incidence of relapse was 20% (95% CI, 13% to 29%) at 1 year, 23% (95% CI, 16% to 33%) at 2 years, 26% (95% CI, 19% to 36%) at 3 years, and 27% (95% CI, 20% to 37%) at 5 years. Twenty of 29 relapse events (69%) occurred during the first year after transplantation, 4 of 29 events (14%) occurred during the second year, 3 of 29 events (10%) occurred during the third year, and 1 event each occurred in years 4 and 8. The median time to relapse was 6 months (range, 2 to 89 months). NRM was 12% (95% CI, 7% to 20%) at 1 year, 16% (95% CI, 10% to 25%) at 2 years, 18% (95% CI, 12% to 27%) at 3 years, and 18% (95% CI, 12% to 27%) at 5 years. Specific causes of NRM included GVHD ( $n = 14$ ), infection ( $n = 4$ ), and toxicity ( $n = 2$ ).

### Classification of Chimerism Status

The median number of chimerism assessments per patient was 6 for peripheral blood and 5 for bone marrow. With regard to CD4<sup>+</sup> cells in peripheral blood, 49% of the 101 analyzed patients had CC, 11% had II-MC, 21% had de-MC, and 19% had in-MC. Classification of chimerism status in the CD8<sup>+</sup> and CD19<sup>+</sup> cell populations was comparable (CD8<sup>+</sup> cells: 42% CC, 17% II-MC, 24% de-MC, and 17% in-MC; CD19<sup>+</sup> cells: 47% CC, 14% II-MC, 22% de-MC, 17% in-MC). In contrast, classification of chimerism status for CD34<sup>+</sup> cells in the bone marrow showed marked differences, with a lower frequency for CC and a higher frequency for de-MC and in-MC (6% CC, 18% II-MC, 32% de-MC, and 44% in-MC).

### Prognostic Impact of Chimerism Status in Peripheral Blood

The cumulative incidence of relapse with regard to chimerism status in peripheral blood CD4<sup>+</sup> cells was similar in patients with CC, II-MC, and de-MC (22% [95% CI, 12% to 43%], 31% [95% CI, 12% to 83%], and 26% [95% CI, 12% to 55%], respectively), but significantly higher for patients with in-MC

(63%; 95% CI, 45% to 89%;  $P = .00072$ ) (Figure 1A). In-MC chimerism status also was a negative prognostic factor for OS, whereas NRM was not significantly increased (Figure 1B and C). In-MC chimerism status remained an independent negative prognostic factor for relapse and OS when analyzed in multivariate Cox regression models that included chimerism status as a time-dependent covariate, in addition to baseline patient and transplantation characteristics as well as the time-dependent covariates administration of DLI and occurrence of aGVHD, late aGVHD, and cGVHD (Table 2).

Results for relapse, NRM, and OS with regard to chimerism status in peripheral blood CD8<sup>+</sup> and CD19<sup>+</sup> cells were comparable to the results for CD4<sup>+</sup> cells, with the exception of II-MC in CD8<sup>+</sup> cells, which was identified as a negative prognostic factor for NRM (Table 2). Importantly, the prognostic impact of chimerism in the CD4<sup>+</sup>, CD8<sup>+</sup>, and CD19<sup>+</sup> subpopulations did not change significantly when chimerism status was analyzed separately for patients with B-ALL and those with T-ALL (data not shown).

### Prognostic Impact of Chimerism Status in Bone Marrow

The cumulative incidence of relapse with regard to chimerism status in bone marrow CD34<sup>+</sup> cells was similar in patients with CC, II-MC, and de-MC (17% [95% CI, 3% to 99%], 17% [95% CI, 6% to 47%], and 14% [95% CI, 4% to 44%], respectively), but significantly higher in patients with in-MC (53%; 95% CI, 38% to 73%;  $P = .0016$ ) (Figure 1D). In-MC chimerism status of CD34<sup>+</sup> cells also was identified as a negative prognostic factor for OS, whereas NRM was not increased significantly (Figure 1E and F). In-MC chimerism status of CD34<sup>+</sup> cells remained an independent negative prognostic factor for relapse and OS in multivariate Cox regression analysis (Table 2).

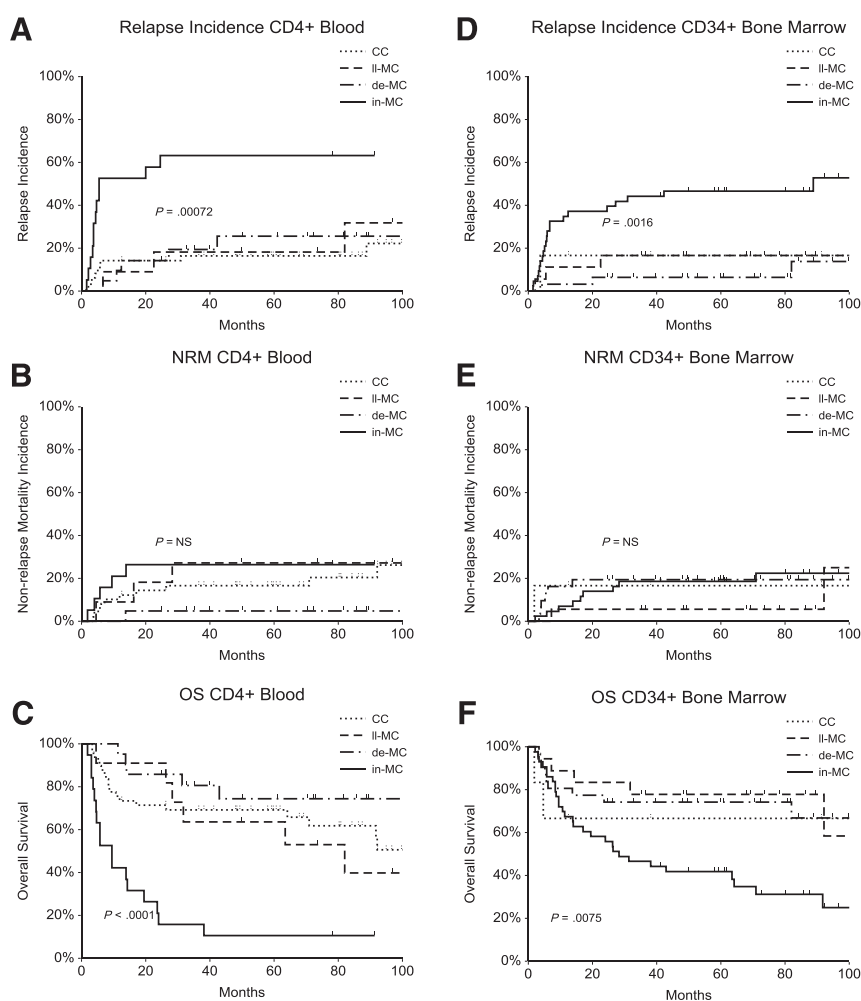
In patients who relapsed, the median time to relapse after detection of in-MC in CD34<sup>+</sup> bone marrow cells was 116 days (range, 0 to 2639 days), which trended to be longer compared with 25 days (range, 0 to 641 days) for detection of in-MC in any of the peripheral blood cell populations ( $P = .091$ ). In addition, the sensitivity for relapse detection by identification of in-MC status in bone marrow was somewhat higher compared with blood (79% [95% CI, 60% to 90%] versus 55% [95% CI, 37% to 72%];  $P = .061$ ), whereas the specificity of chimerism assessment in bone marrow was significantly lower (70% [95% CI, 58% to 77%] versus 87% [95% CI, 78% to 93%];  $P = .012$ ).

### Prognostic Impact of MRD Status in Bone Marrow

Serial pretransplantation and post-transplantation qPCR MRD data were available for a subgroup of 22 patients, of whom 2 were monitored for BCR-ABL and 20 were monitored for Ig/TCR gene rearrangements. The median number of MRD assessments per patient was 5, comparable to the number of assessments for chimerism in peripheral blood and bone marrow.

With regard to MRD status before transplantation, 8 of 22 patients were MRD-negative, and the other 14 were MRD-positive. After transplantation, 2 of the 8 patients who were initially MRD-negative changed to MRD-positive, whereas 12 of the 14 initially MRD-positive patients achieved at least temporary MRD negativity.

With regard to the predictive value of MRD after transplantation, 7 of the 22 patients experienced MRD relapse or MRD persistence after transplantation, whereas the other 15 patients became and/or remained MRD-negative. The cumulative incidence of hematologic relapse in patients with



**Figure 1.** (A–C) Cumulative incidence of relapse (A), NRM (B), and OS (C) according to chimerism status in CD4<sup>+</sup> peripheral blood T cells in 101 patients undergoing HCT for ALL. (D–F) Cumulative incidence of relapse (D), NRM (E), and OS (F) according to chimerism status in CD34<sup>+</sup> bone marrow cells in these 101 patients.

MRD relapse was 86% (95% CI, 63% to 100%), compared with only 7% (95% CI, 1% to 44%) in patients who remained MRD-negative ( $P = .0035$ ) (Figure 2A).

Post-transplantation MRD relapse also was prognostic for OS, but not for NRM (Figure 2B and C), and it remained an independent prognostic factor for relapse and OS when analyzed in a multivariate Cox regression model with MRD status as a time-dependent covariate (Table 2).

The median time to hematologic relapse after detection of MRD relapse was 173 days (range, 0 to 2373 days), somewhat longer than the interval reported for chimerism. MRD assessment detected relapse with high sensitivity (86%; 95% CI, 49% to 97%) and high specificity (95%; 95% CI, 70% to 99%), comparing favorably with the respective values for chimerism.

With regard to the prognostic impact of chimerism assessment in the subgroup of patients with available MRD data, 4 of the 7 patients with MRD relapse and subsequent hematologic relapse also would have been detected via a signal of in-MC, with 3 of these detections occurring earlier than MRD, but the other 3 patients would have been missed by relying on the in-MC signal only. Among the 14 patients without MRD relapse and without hematologic relapse, 9 patients also would have been negative for in-MC, but the other 5 patients would have generated a false-positive in-MC signal.

### Extramedullary Relapse

Among the overall 29 cases of relapse, 3 patients had isolated extramedullary relapse, and 6 patients had combined extramedullary and bone marrow relapse. Two of the 3 patients with isolated extramedullary relapse had an in-MC signal in bone marrow and blood before relapse, whereas the other patient had an in-MC signal in blood only. None of these 3 patients had available MRD data, and thus we could not evaluate whether MRD would have been predictive as well. One of the 6 patients with combined extramedullary and bone marrow relapse had an in-MC signal in bone marrow and blood before relapse, whereas the other 5 patients had an in-MC signal in bone marrow only. Thus, based on this limited experience, chimerism analysis in bone marrow was able to predict relapse in 8 of 9 cases of extramedullary relapse (89% sensitivity), whereas chimerism analysis in blood was able to predict relapse in only 4 of 9 cases (44% sensitivity), and chimerism analysis in bone marrow or blood was able to predict relapse in 9 of 9 cases (100% sensitivity).

### Impact of DLI on Chimerism and MRD

Sixteen patients had received preemptive DLI for MC, and 12 of these 16 patients converted to CC after DLI. At last follow-up, 10 patients in this cohort remained alive in CR.



**Table 2**

Multivariate Cox Proportional Hazard Regression Analysis Estimating the Effect of Chimerism and MRD Status on Relapse, NRM, and OS

	Relapse		NRM		OS	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
CD4 <sup>+</sup> blood						
CC	1		1		1	
II-MC	1.71 (0.46–6.36)	.42	3.68 (0.82–16.40)	.088	2.49 (0.88–7.01)	.085
de-MC	0.84 (0.25–2.81)	.78	0.24 (0.03–1.88)	.17	0.51 (0.19–1.37)	.18
in-MC	5.32 (2.10–13.45)	<.001	4.61 (0.91–14.88)	.063	4.17 (2.05–8.51)	<.001
CD8 <sup>+</sup> blood						
CC	1		1		1	
II-MC	0.91 (0.25–3.33)	.89	4.24 (1.35–13.37)	.014	2.14 (0.99–4.63)	.053
de-MC	0.46 (0.14–1.53)	.21	0.19 (0.02–1.49)	.113	0.444 (0.16–1.21)	.11
in-MC	4.15 (1.64–10.51)	.003	3.38 (0.84–13.67)	.087	4.19 (1.99–8.86)	<.001
CD19 <sup>+</sup> blood						
CC	1		1		1	
II-MC	0.61 (0.17–2.25)	.46	0.85 (0.18–4.03)	.84	0.96 (0.35–2.62)	.93
de-MC	0.52 (0.17–1.61)	.25	0.18 (0.02–1.43)	.11	0.53 (0.20–1.44)	.21
in-MC	4.10 (1.55–10.80)	.004	5.13 (0.96–27.37)	.056	3.65 (1.71–7.81)	.001
CD34 <sup>+</sup> bone marrow						
CC	1		1		1	
II-MC	1.14 (0.26–4.98)	.86	2.72 (0.23–31.62)	.42	3.07 (0.58–16.31)	.19
de-MC	0.46 (0.11–1.98)	.29	3.90 (0.46–33.43)	.21	3.22 (0.68–15.24)	.14
in-MC	3.58 (1.26–10.191)	.017	7.03 (0.86–57.63)	.069	8.99 (2.11–38.31)	.003
MRD bone marrow						
Negative	1		1		1	
Relapse	24.64 (1.58–384.19)	.022	0.04 (0–13074423)	.75	9.67 (1.93–48.50)	.006

HR indicates hazard ratio.

Prognostic variables examined were transplant year, sex, age, Karnofsky Performance Status, HCT-CI, disease status, lineage, cytogenetic risk group, donor type, stem cell source, ATG infusion, and the time-dependent covariates application of DLI, occurrence of aGVHD, late aGVHD, and cGVHD, chimerism status, and MRD status. Only results for chimerism and MRD status are shown here. Other variables with significant prognostic impact in the multivariate setting were disease status and occurrence of cGVHD for relapse and OS and HCT-CI for NRM. HR and *P* values refer to the comparison of the respective category with the first one.

Two patients had received preemptive DLI for recurrent or persistent MRD, and both returned to molecular negativity after DLI. These patients experienced a prolonged relapse-free interval after DLI but still sustained late relapse, and both died from leukemia (at 1764 days and 2322 days after DLI).

Thirteen patients had received prophylactic DLI because of active disease at time of transplantation; 3 of these patients had MC at the time of DLI, 1 of whom converted to CC after DLI. At last follow-up, 8 patients in this cohort remained alive in CR.

Seven patients were treated with DLI for overt relapse after receiving additional reinduction therapy. All 7 patients had MC at time of DLI, and 2 of the 7 converted to CC after DLI. At last follow-up, only 1 patient in this cohort remained alive in CR.

## DISCUSSION

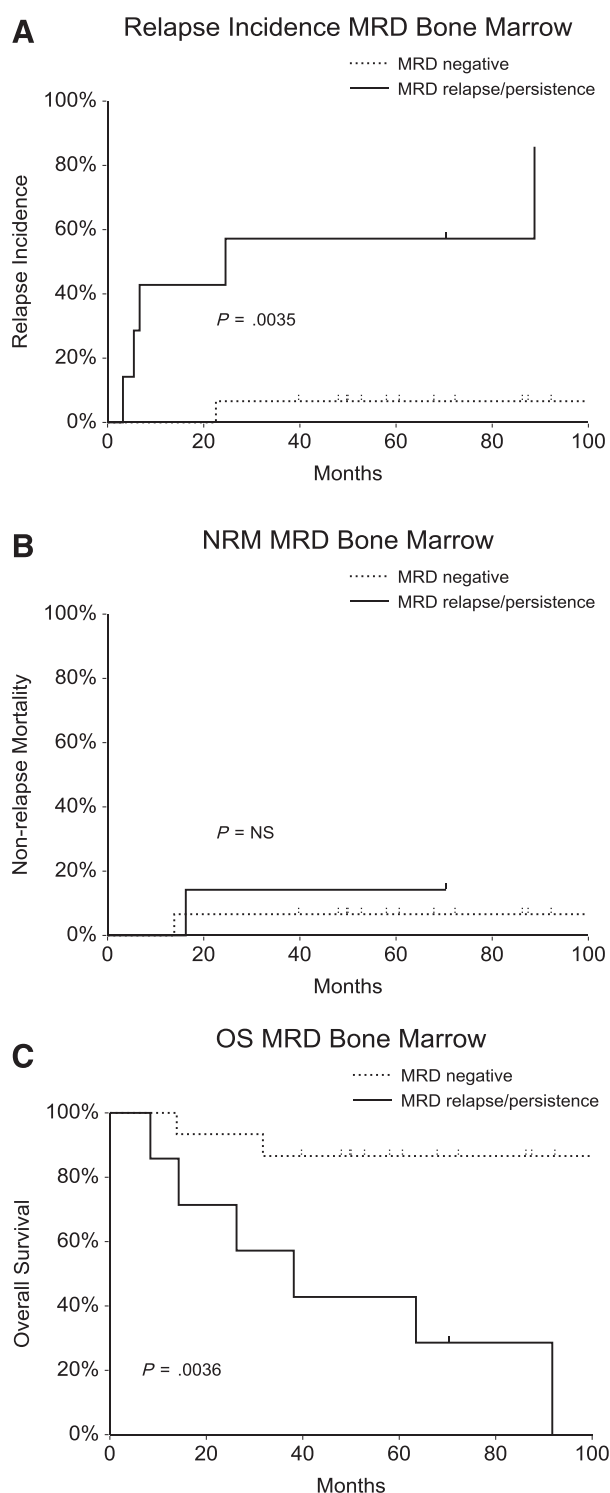
MRD monitoring has become an integral part of many protocols for initial chemotherapy of patients with newly diagnosed ALL [10,12,44], but it is much less frequently applied after transplantation, where chimerism monitoring provides an alternative for early relapse prediction. Although MRD presumably should perform better with regard to this goal, very limited comparative data actually have been reported, and no reference method has yet been defined [27,34,45,46].

The present study provides evidence in an adult ALL population that the higher in vitro sensitivity of MRD testing and the specificity for leukemic clones translates directly into the clinical setting, with earlier detection of imminent relapse and greater sensitivity and specificity compared with chimerism. Owing to low patient numbers, we could not determine whether such improved detection actually

resulted in improved outcomes; however, previous studies indicate that therapeutic intervention at low MRD levels is advantageous [7,8].

In more detail, our chimerism analysis showed that only in-MC was associated with increased relapse incidence, whereas CC, II-MC, and de-MC had no differential effect on outcome. These data confirm previous studies in pediatric ALL [20] and in adult mixed ALL and AML populations [47]. The considerable difference in prognostic impact between de-MC and in-MC clearly indicates that chimerism is a dynamic marker, warranting the analysis of at least 2 time points before therapeutic interventions are considered. The dynamic aspect of chimerism also explains why previous studies that assessed only CC and MC categories and not the subcategories II-MC and de-MC failed to identify any prognostic relevance [48,49].

The finding that II-MC had no adverse effect on outcomes compared with CC confirms that stable mixed chimerism at low levels is compatible with prolonged DFS [20,50], but also raises the question of whether methodologies for chimerism assessment with a sensitivity below the 1% to 5% level really have greater prognostic value in clinical applications. Despite the fact that various studies have described highly sensitive detection of potentially malignant cells by confining chimerism analysis to the leukemic phenotype [22,27,51], no data are available indicating that such an approach actually improves relapse prediction [18]. In fact, a recent report by Zeiser et al. [52] concluded that lineage-specific chimerism analysis has no better sensitivity and specificity for hematologic relapse than the conventional approach, despite a 20- to 30-fold greater in vitro sensitivity. Likewise, in our study, chimerism analysis in the CD4<sup>+</sup>, CD8<sup>+</sup>, and CD19<sup>+</sup> subpopulations had no differential prognostic value, even when analyzed separately for patients with B-ALL and patients



**Figure 2.** Cumulative incidence of relapse (A), NRM (B), and OS (C) according to MRD status in bone marrow in 22 patients undergoing HCT for ALL.

with T-ALL. Thus, it is possible that chimerism monitoring at 1% to 5% sensitivity without selecting for leukocyte subsets is sufficient for clinical purposes.

With regard to the prognostic value of in-MC status in bone marrow versus peripheral blood, we found that bone marrow had somewhat better sensitivity for relapse detection (79% versus 55%), with a trend for an earlier signal, but

lower specificity (70% versus 87%). Most previous studies focused on either blood or bone marrow, or did not provide comparative analyses [20,45,53], and thus this inverse association of specificity and sensitivity of blood and bone marrow has not been reported previously. A possible explanation for the low specificity of in-MC in the bone marrow is contamination with normal stromal cells, which have been shown to remain entirely of recipient origin after HCT [54], and can express CD34 [55]. Another possible contributing factor is that ALL clones can be CD34<sup>+</sup> at diagnosis [56], or that CD34 can be lost at relapse [57,58]. In both of these situations, any CD34 host signal is of purely benign origin, causing the lower specificity of this test.

With regard to MRD assessment, our study found that relapse prediction by MRD was much more sensitive (86%) and specific (95%) than prediction by chimerism analysis. In fact, only 1 of the 22 patients with available MRD data experienced hematologic relapse without a previous MRD signal. This patient had been MRD-positive before transplantation, but achieved MRD negativity at day +28 and day +117 while in II-MC. Because the patient's chimerism improved to CC at day +251, no further MRD monitoring was performed. Stable CC was confirmed at days +341 and +600; however, at day +684, the patient experienced hematologic relapse. The foregoing case, along with our finding that 31% of relapses occurred beyond 1 year and that 17% of patients with CC still experienced relapse, as well as the report from Rossi et al. [45] of up to 30% MRD positivity in patients with CC, strongly suggest the importance of continuing MRD measurements for longer periods even when CC has been achieved.

MRD positivity after transplantation was associated with the highest relapse risk of all prognostic groups analyzed, even when conventional risk factors, such as disease status at transplantation, or post-transplantation events, such as occurrence of GVHD, were considered in a multivariate setting. The median time from first MRD detection to relapse was 173 days, which is in line with previous observations [30] and indicates an opportunity for timely initiation of therapeutic interventions [59,60]. In fact, the sole patient who did not relapse despite MRD positivity was a patient with Philadelphia chromosome-positive ALL, who was treated successfully with tyrosine kinase inhibitor therapy. Importantly, despite the inferior overall performance of chimerism assessment in our overall analysis and in the subgroup of patients with available MRD data, 3 of 7 MRD-positive relapse cases had a chimerism signal even before MRD, indicating that a combined assessment of these 2 modalities is still useful.

Our data on DLI support previous studies demonstrating the ability of preemptive and prophylactic DLI to convert MC to CC, possibly preventing relapse [20,39,61]. Additional data regarding a protective effect of DLI in ALL also come from an earlier study demonstrating that DLI-induced GVHD is associated with a potent graft-versus-leukemia effect [38]. Therapeutic DLI in patients with active disease seems to have only a limited benefit, however [38,62].

An important limitation of the present study is the potential for bias owing to possible differences in the groups analyzed. However, with the exception that patients with available MRD data underwent transplantation more recently and had greater use of ATG compared with patients without MRD data, all other characteristics were similar, including the frequency of DLI. Another potential confounder is that the administration of DLI, affecting the natural history

of the disease, could have influenced our results. However, application of DLI was included in our multivariate Cox proportional hazards regression model and was found to have no significant impact on the prognostic value of chimerism and MRD assessment. In addition, we applied our multivariate Cox proportional hazards regression model separately for patients who received DLI and those who did not, and in that sensitivity analysis, in-MC status and MRD positivity remained relevant prognostic factors in both patient cohorts (data not shown). Finally, although the small number of patients monitored via qPCR for BCR-ABL did not allow analysis of the relative performance of this assay, and although some bias might have been introduced because of the availability of tyrosine kinase inhibitor as a potential treatment for MRD relapse, we felt that including these patients was important to maintain the comparability of patient characteristics in the chimerism and MRD patient cohorts.

In conclusion, in light of recent advances in therapeutic options for post-transplantation relapse [7,8,63,64], improving our understanding of the available relapse prediction tools is becoming increasingly important. Our study demonstrates the superiority of MRD over chimerism analysis in this regard, clearly indicating that MRD should be incorporated into the routine post-transplantation monitoring of all patients with ALL [11,16,17,23]. Chimerism analysis remains a valuable tool for monitoring engraftment, especially in the setting of reduced-intensity conditioning [65], as well as for patients without a suitable MRD marker [22]. In select cases, chimerism assessment may predict relapse earlier than MRD, and it also may be useful for predicting extramedullary relapse, but good clinical judgment accounting for the dynamic aspects of chimerism is required. Standards for measurement intervals for MRD and chimerism and definitions of thresholds for initiating therapy are still missing. Ultimately, prospective studies are needed to determine the optimal use of these tools with the aim of facilitating risk-adapted therapeutic interventions.

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